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Arthrobacter species as a prey cell reservoir for nonobligate bacterial predators in soil[1]

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The possibility was investigated that, in soil, *Arthrobacter* species might serve as a major reservoir of prey cells for the nonobligate bacterial predators in the soil. Previous evidence had indicated this. *Arthrobacter globiformis* cells added to soil caused an increase in the total bacterial count and the gram-negative bacteria count of the soil. Copper-resistant bacterial predators, such as *Cupriavidus necator*, also increased in number, apparently in response to the *A. globiformis* cells. Other bacterial predators did not respond to *A. globiformis*. Certain soil bacteria responded specifically and quickly (within 2.5 h) to the *A. globiformis* cell additions. They had gliding motility and could hydrolyze GELRITE (the solidifying agent for media). Addition of these hydrolyzer bacteria to soil caused marked increases in the total bacteria count, the gram-negative bacteria count, and the bacterial predator counts. These responses mimicked those for *A. globiformis* soil additions. The results from an alternative method of soil incubation that speeded up the processes, and from other observations, indicated that the large apparent bacterial predator attack on *A. globiformis* in soil may actually be on other bacteria in soil that respond to *A. globiformis* in a nonpredatory manner. Therefore, *A. globiformis* and other *Arthrobacter* species may not be serving as a major reservoir of prey cells in soil.

Key words: predation, predators, prey, soil, *Arthrobacter*.

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Une investigation a été entreprise sur la possibilité que, dans les sols, des espèces d'*Arthrobacter* puissent servir de réservoir majeur de cellules de proie pour des prédateurs bactériens non-obligés des sols. Des résultats antérieurs avaient indiqué cette possibilité. L'ajout dans des sols de cellules d'*A. globiformis* a causé une augmentation du nombre total de bactéries et de celui des bactéries Gram négatives dans ces sols. Des prédateurs bactériens résistants au cuivre, comme le *Cupriavidus necator*, ont aussi augmenté en nombre, apparemment en réponse à la présence des cellules d'*A. globiformis*. D'autres prédateurs bactériens n'ont pas répondu à la présence d'*A. globiformis*. Certaines bactéries du sol ont reconnu spécifiquement et rapidement, soit en dedans de 2,5 h, à l'ajout de cellules d'*A. globiformis*. Ces bactéries, dont la motilité s'operait par glissement, ont pu hydrolyser le GELRITE (un agent de solidification des milieux de culture). L'addition de ces bactéries hydrolysantes dans des sols a causé des augmentations marquées dans le nombre total de bactéries et dans celui des prédateurs bactériens. Ces réponses concordaient avec celles d'additions de cellules d'*A. globiformis* dans les sols. Des résultats obtenus par une méthode alternative d'incubation permettant d'activer les processus, appuyés par d'autres observations, ont indiqué que l'ampleur apparente de l'attaque des prédateurs bactériens sur l'*A. globiformis* dans les sols pourrait bien avoir été exercée sur d'autres bactéries des sols qui répondent à la présence d'*A. globiformis* d'une façon autre que par prédation. Conséquemment, il est possible que l'*A. globiformis* et d'autres espèces d'*Arthrobacter* ne servent pas de réservoir majeur de cellules de proie dans les sols.

Mots clés : prédation, prédateurs, proie, sol, *Arthrobacter*.

[Traduit par la revue]

Introduction

Cells of *Arthrobacter* species occur in large numbers in soil. In fact, some workers consider that these bacteria constitute the largest bacterial population that is detectable in soil by conventional bacteriological procedures (Boyien 1973). *Arthrobacter globiformis* is an example of these soil *Arthrobacter* species. In soil, *A. globiformis* is attacked by indigenous cells of the bacterial predators, *Cupriavidus necator* (Makkar and Casida 1987*b*) and strain L-2, but not by some other less powerful predators (Zeph and Casida 1986). *Cupriavidus necator* and strain L-2 are representatives of the copper-resistant bacterial predators (Casida 1988). These are nonobligate predators, and they can attack an array of other bacterial species, including other bacterial predators. From the foregoing, it would appear that soil *Arthrobacter* species can serve as a large reservoir of potential prey cells for the copper-resistant bacterial predators. If true, then fluctuations in *Arthrobacter* species numbers in soil should affect the numbers of the predator bacteria. This in turn should affect the numbers of other potential prey cell species for these predators. For example, addition to soil of unique nutrients for *Arthrobacter* species could activate them or even increase their numbers, followed by increased predator numbers. The latter would not only attack *Arthrobacter* species, but also attack any other prey species in the vicinity. This predator response would be somewhat like that proposed by Mallory *et al.* (1983) for protozoa when they have alternative prey bacteria available.

The present study was undertaken to determine whether, in soil, *Arthrobacter* species do serve as a huge prey cell reservoir for bacterial predators such as *C. necator* and related types. The alternative possibility examined was whether the predator attack that is seen might, in part, be an indirect response to other soil bacteria that respond in a nonpredatory fashion to *Arthrobacter* species.

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Materials and methods

Organisms and media

Arthrobacter globiformis (ATCC 8010), *Cupriavidus necator* (ATCC 43291) (Makkar and Casida 1987*b*), and strain L-2 (Zeph and Casida 1986) were maintained on slants of 0.1 strength heart infusion agar. Strain L-2 was a copper-resistant, nonobligate bacterial predator of bacteria in some ways resembling *C. necator*. Washed spore and (or) crystal suspensions of *Bacillus thuringiensis* (H-type 3a3b-2) were prepared as described by Petras and Casida (1985).

Washed-cell suspensions of *A. globiformis* were prepared for cells grown in heart infusion broth (full strength), tryptic soy broth, or synthetic media. The media were dispensed as 40 mL per 300-mL baffle-bottom flask. Modified N-1 synthetic medium contained 0.1% NH_4Cl, 0.1% dextrose, 0.1% KH_2PO_4, 0.02% Na_2SO_4, and 0.02% NaCl, pH 7.0, adjusted with KOH. AG synthetic medium contained 0.25% KH_2PO_4, 0.02% Na_2SO_4, 0.02% NaCl, 0.1% KNO_3, 0.02% $MgCl_2 \cdot 6H_2O$, 0.02% $CaCl_2 \cdot 2H_2O$, 0.01% $FeCl_3 \cdot 6H_2O$, and 0.25% dextrose, pH 7.4, adjusted with KOH. The GELRITE (Lin and Casida 1984) hydrolyzers were grown in tryptic soy broth, in GELRITE broth, or on milk agar. The GELRITE broth was full-strength heart infusion broth containing 0.2% GELRITE, but without added magnesium. The GELRITE was broken up with a sterile glass rod just before inoculation. The inoculated flasks for the various media were shaken for 2–3 days at 27°C before washing the cells. Milk agar was placed in petri plates. It comprised nutrient agar to which had been added 0.1% yeast extract and 1.0% nonfat dehydrated milk powder. After incubation of the plates for 5 days, the cells were gently scraped from the agar surface with 5 mL of double-distilled water, then washed. All except the synthetic media were Difco products (Difco Laboratories, Detroit, MI).

Soil incubations

Soils RS84, RS85, and RS86 were a Hagerstown silty clay loam. They were obtained from different fields and at different times from a farm near University Park, PA. The soil pH values were 6.3, 4.9, and 5.5, respectively.

The soils were incubated in various ways. For the soil-bottle procedure, the soil was passed through a 3-mm sieve, then 10-g portions were added to sterile 1-ounce (ca. 29.6 mL) screw-cap bottles. Sterile water (1.2 mL), washed-cell suspension, or chemical solution were added to bring the soil to 60% of its moisture-holding capacity (MHC). The caps were left partially loose during incubation. Incubation usually was for 3 days at 27°C. Longer incubations of the soil were involved when sequential additions of washed *A. globiformis* cells were made. At time zero, 1.2-mL portions of washed cells or water were added to several bottles of soil. Further additions of 0.3-mL portions of cell suspension or water were made at 4, 7, 11, 19, and 25 days. Therefore, some of the bottles that had received cells at time zero received water at all succeeding times, while others received additional cells. Other bottles received only water at all times, including time zero.

Soils were also incubated by shaking for 24 h with added water or cell suspensions. For these trials, 1 g of soil was placed in a sterile tube with 9 mL of sterile distilled water. Two drops of washed cell suspension were added. The soil plus water and cells in the tube were then mixed thoroughly with a Vortex Genie Mixer (American Hospital Supply Corporation, Evanston, IL) and poured aseptically into a sterile 500-mL Erlenmeyer flask. This was shaken 24 h at 27°C. Further dilutions for plating were then prepared from this 10^{-1} dilution.

The washed-cell suspensions that were added to soil provided numbers of cells per g of soil as follows. For *A. globiformis*, the numbers were 1.0×10^7 for cells grown in nutrient broth, and 9.0×10^9 for cells grown in tryptic soy broth. GELRITE hydrolyzer strains 487 and GF3RP grown in tryptic soy broth gave 4.0×10^7 and 4.0×10^6, respectively, per g of soil. The GF3RP count possibly is low because of difficulty in counting them. The *B. thuringiensis* spores gave 3.9×10^8 per g of soil.

Microbial enumeration

Soil dilutions were made in 10-fold increments in tubes containing 9 mL of sterile distilled water. Each tube was thoroughly mixed with a vortex mixer before 1 mL was removed for preparing the next dilution of the sequence. The dilutions were plated on various media. Colony numbers for copper-resistant bacteria and fungi were determined on 0.1 strength heart infusion agar containing 0.01% $CuCl_2 \cdot 2H_2O$ (pH 6.5). Colony numbers for total bacterial counts, whether copper resistant or not, were determined using a medium containing 0.01 strength heart infusion broth, 0.01% L-alanine, 0.07% $MgCl_2 \cdot 6H_2O$, and 0.6% GELRITE (Lin and Casida 1984) to solidify the medium. It was important that the $MgCl_2 \cdot 6H_2O$ be dissolved completely before adding the GELRITE. The medium was poured at 60°C into petri plates. Gram-negative bacteria, whether copper resistant or not, were enumerated on a similar medium but with crystal violet added to give 4.9 μM before adding the GELRITE.

All incubations of soils and plates were at 27°C. The plates were incubated 5 to 6 days.

The numbers in soil of *C. necator* and L-2 cells were estimated by the method of Makkar and Casida (1987*a*).

The soil enumeration results are presented in 3 ways. Some of the results are stated as the actual numbers of organisms that were present at the sampling. Other results are presented as the ratio (relative increase in numbers) of the count at a given time for soil incubated with added cells, as compared with the control soil incubated for the same period but with only water added. Lastly, some of the results are presented as the ratio of the counts at a given time during incubation to the count that was present at time zero. This allowed the control values to be shown where only water was added to the soil before it was incubated.

Results

Natural (nonsterilized) soil was incubated 2 ways for these trials. In order to roughly approximate its state in nature, soil was placed in bottles and adjusted to 60% of MHC for incubation. Alternatively, to speed up microbial activity, soil was submerged in water and incubated shaken in a flask.

Soil bottle incubations

Arthrobacter globiformis was incubated 3 days in bottles containing 10 g of soil adjusted to 60% of MHC. The controls received water instead of *A. globiformis* cells. Table 1, based on six repeat experiments with three soils (two for each soil), shows that the total bacterial count and the gram-negative bacterial count of the soils responded strongly to *A. globiformis* addition. Smaller responses, however, also occurred for the bacterial predators, *C. necator* and strain L-2. The numbers of the *A. globiformis* cells in the soil decreased only slightly during the incubation. This was shown in other trials that extended over a greater period of time. For example, 9.4×10^9 cells/g soil at time zero was 5.3×10^8 at 6 days and 2.2×10^8 at 3 weeks.

The nature of the predator bacteria responses was evaluated by making sequential additions of *A. globiformis* to the soil, as opposed to just one, initial addition. The responses of indigenous *C. necator* and strain L-2 cells in the soil are seen in Figs. 1 and 2. It would appear that making several additions of *A. globiformis* to soil did not to any extent increase the *C. necator* or strain L-2 count beyond that for a single addition. No increases occurred if only water was added.

Glutamic acid (1.2 mL of a 1% solution) was added to soil instead of adding *A. globiformis* cells. As a result, the numbers of *C. necator* and strain L-2 in the soil each rose to 1.3×10^6/g soil within 3 days. Their initial numbers in the soil were 1.3×10^2 and 2.3×10^2, respectively.

TABLE 1. Relative increases in bacterial counts for soil incubated 3 days at 60% of MHC with added *A. globiformis* cells as compared with control soil incubated with only added water

Bacteria	Increase in count, fold
Total count[a]	96(20–306)
Gram negative	295(3–862)
C. necator	4
L-2	10

NOTE: The values for total count and gram negative are averages for six experiments with the three soils (two per soil). The ranges for the values are shown within the parentheses. The *C. necator* and strain L-2 values are for the RS84 soil.

[a]Residual of *A. globiformis* cells, or any multiplication of them, was not a component of the total count.

Occurrence of hydrolyzers

Total-bacteria counts made on GELRITE medium (no crystal violet) for soils that had been incubated with added *A. globiformis* cells showed some small, concave depressions on the surface of the medium. In general, they were about 2 to 7 mm in diameter and 1 to 3 mm deep. In some cases, however, they extended down through the medium to the glass bottom of the petri plate. The organisms that caused these depressions did not necessarily produce a defined colony at the bottom of the depression. Separate experiments, not reported here, showed that these organisms were able to hydrolyze GELRITE.

The relation of GELRITE hydrolysis to *A. globiformis* was studied. *Arthrobacter globiformis* was grown in tryptic soy broth, full-strength heart infusion broth, modified N-1 synthetic medium, or AG synthetic medium. The cells were washed, added to bottles containing either RS84 or RS85 soil, and incubated for 3 days. Dilutions of the soil then were plated for total counts on GELRITE medium. Incubation of the RS84 soil (pH 6.3) with *A. globiformis* cells yielded 2.6×10^6 (SD $\pm$ 2.5×10^6) GELRITE-hydrolyzing cells/g of soil. Incubation with the RS85 soil (pH 4.9) gave 3.9×10^5 (SD $\pm$ 3.6×10^5). Only occasionally did a GELRITE hydrolyzer occur in the absence of *A. globiformis*. For these counts, the depressions in the GELRITE medium were apparent regardless of the presence of other bacterial colonies on the low-dilution plates. Growth of *Streptomyces* species and *Bacillus mycoides* from the soil did not interfere because these organisms, in some manner, were held in check when *A. globiformis* was added to soil. The choice of the medium for the initial growth of *A. globiformis* to obtain the washed cells did not affect the results. The culture filtrates obtained from growth of the *A. globiformis* cells in either of the synthetic media did not cause growth of the GELRITE-hydrolyzing organisms when the filtrates were added to the soil. This was also true for a 10-fold concentrate (rotary vacuum evaporator) made of the modified N-1 synthetic medium filtrate. Therefore, the activity apparently resided with the cells themselves. In three separate trials, *A. globiformis* cells that had been autoclaved before addition to the soil retained 3, 19, and 69%, respectively, of their ability to stimulate growth of the GELRITE-hydrolyzing organisms in the soil.

In addition to *A. globiformis*, only *Micrococcus luteus* stimulated the GELRITE hydrolyzers. To show this, washed cells of various bacteria (grown in full-strength heart infusion broth) were incubated 3 days in RS84 soil. *Arthrobacter globiformis* gave 2.0×10^6 GELRITE-hydrolyzing organisms/g soil, and *M. luteus* gave 7.0×10^5. There was no hydrolyzer organism response to addition to soil of *Agrobacterium tumefaciens*, *Agromyces ramosus*, *Nocardia salmonicolor*, or *Escherichia coli*. There also was no response to *Bacillus thuringiensis* spores and (or) crystals, or to strains 50 or GF3RP cells (see later) grown on milk agar or in GELRITE broth. Note that neither *A. globiformis* nor *M. luteus* can hydrolyze GELRITE on their own.

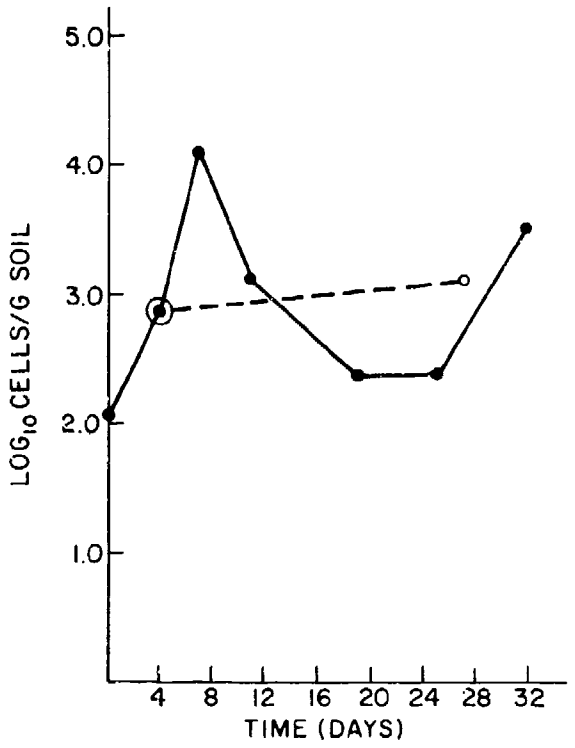

FIG. 1. Growth response of indigenous *C. necator* cells in soil to sequential additions of washed *A. globiformis* cells. The cell additions were made at time zero without further additions (○), or at time zero but with further additions (cumulative) also made at each of the sampling times except the final one at 32 days (●).

Addition of aqueous solutions of various compounds, instead of *A. globiformis*, to provide 1.0 mg/g soil did not cause the appearance of GELRITE-hydrolyzing organisms. The compounds tested were GELRITE, dipicolinic acid, arginine, tryptophan, threonine, and isoleucine.

The response of the indigenous GELRITE-hydrolyzing cells in soil to addition of washed *A. globiformis* cells (grown in tryptic soy broth) occurred very quickly. GELRITE-hydrolyzing organisms were not present (10^{-4} dilution of soil) at time zero, 1 h, and 3 weeks. They were detected, however, after a 2.5-h soil incubation (3.6×10^5/g soil) and after 6 days (2.0×10^5/g soil).

Soil response to hydrolyzer isolates

As noted above, various GELRITE-hydrolyzing bacteria showed up when *A. globiformis* cells were incubated in soil. Three representatives of these bacteria were isolated for further study. They were designated as strains 50, 487, and GF3RP. Strain 50 hydrolyzed GELRITE on isolation from soil, but lost the ability during later transfers on media not containing GELRITE. The other isolates retained the ability.

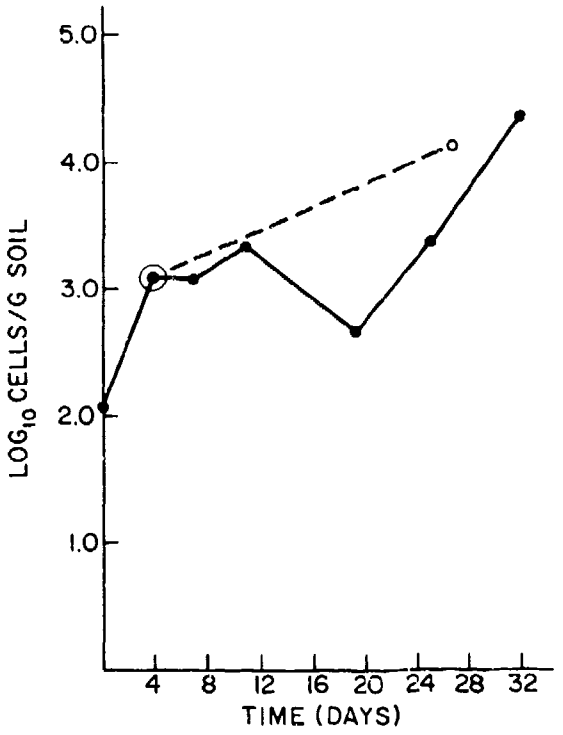

FIG. 2. Growth response of indigenous strain L-2 cells in soil to sequential additions of washed *A. globiformis* cells. The cell additions were made at time zero without further additions (○), or at time zero but with further additions (cumulative) also made at each of the sampling times except the final one at 32 days (●).

The three isolates were gram-negative rods that demonstrated gliding motility. Their taxonomic position was unclear, however.

Strains GF3RP, 50, and 487 were added to soil in bottles and incubated for 3 days. The results were approximately the same regardless of which of these organisms was chosen or which soil was used. For eight experiments, the total bacteria count of the soil increased 33-fold more (range = 18–68) when the organism was added than it did for soil incubated with only water added. The gram-negative bacteria count of the treated soil increased 516-fold more (range = 140–950) than it did for the water-control soil. These results were obtained regardless of the medium used for growth of the hydrolyzers (i.e., milk agar, GELRITE broth, or tryptic soy broth). Autoclaving of the hydrolyzer culture cells before incubation in the soil also did not change the results. Although two of the three hydrolyzers could still hydrolyze GELRITE, addition of 1 mg GELRITE (dry powder) per gram of soil along with addition of cells of the hydrolyzer organism did not affect the soil total bacteria counts or gram negative counts attributable to the hydrolyzers. Also, addition to soil of GELRITE by itself without adding the hydrolyzer organism did not increase the soil counts.

In comparing various experiments in which the hydrolyzer bacteria of *A. globiformis* had been incubated 3 days in soil bottles, it appeared that the results were similar. For example, a direct comparison of *A. globiformis* and strain 50 in the same experiment showed the following. Relative to the results for soil incubated with only water added, the increase in total bacteria was 68-fold greater with strain 50 addition, and 87-fold with *A. globiformis*. The gram negative count increased 931-fold with strain 50 and 861-fold with *A. globiformis*.

Shaken flask incubations

Various bacteria were added to soil that had been placed in flasks in an excess of water. The soil was then incubated by shaking the flasks for 24 h at 27°C. This was done to speed up the interaction of predator cells with prey cells. It was also done to increase the rate at which the soil total bacteria count and gram-negative bacteria count would respond to the organisms added to the soil. Finally, it was done to decrease the amount of time available for the occurrence of sequential events in the soil. An example of the latter was bacterial multiplication that could not occur until the multiplication of other bacteria had occurred. These goals seem to have been achieved (see Table 2). Note that the results are stated slightly differently than before, i.e., the results are stated as the relative increase in cell numbers after incubation as compared with the value at time zero. This allowed the control values to be shown where only water was added to the soil before it was incubated.

With the soil incubations carried out in this manner (Table 2), the soil total bacteria counts and gram-negative bacteria counts in response to the GELRITE hydrolyzers (in this case GF3RP and 487) no longer were the same as for the responses to *A. globiformis*. The incubation time period (24 h total) may have been too short to allow the buildup in soil bacterial numbers when starting with *A. globiformis*, but not when starting with the GELRITE hydrolyzers. There was a strong buildup in the numbers of the bacterial predators, i.e., *C. necator*, strain L-2, and the other copper-resistant types. These predators increased in number in response to both *A. globiformis* and the GELRITE hydrolyzers. Simultaneously with this, the numbers of the GELRITE hydrolyzers decreased, as would be expected if they were being attacked by the predators. The numbers of *A. globiformis*, however, did not decrease. In fact, a five-fold increase was observed. A possible interpretation is that *A. globiformis* was not being attacked by the predators to any extent.

Discussion

In preliminary studies (results not reported), use of agar plates with the overstreak procedure of Casida (1988) showed that the growth of *A. globiformis* was not affected by being admixed with various copper-resistant bacterial predators, including *C. necator* and strain L-2. These trials, however, were on agar plates and not in soil. For organisms actually residing in soil, arguments can be raised for considering that *A. globiformis* is a major, if not the major, source of prey cells for nonobligate bacterial predators of bacteria in soil. First, of course, is the conclusion (Boylen 1973) that *Arthrobacter* species comprise the largest group of bacteria in soil, at least for those that can be isolated. In addition, it was shown by Zeph and Casida (1986) and the present study that indigenous cells of the copper-resistant bacterial predators, including *C. necator* and strain L-2, increased in number in soil in response to added *A. globiformis* cells; other less powerful predators did not respond (Zeph and Casida 1986). This increase in predator cell number, however, could not be increased further. For example, sequential additions of *A. globiformis* cells showed a buildup in numbers of *C. necator* and strain L-2 after the first addition of *A. globiformis* cells, but

TABLE 2. Relative increases in cell numbers for various indigenous soil bacterial populations during a 24-h incubation with washed cells of *A. globiformis*, strain GF3RP, or strain 487

Bacteria	Time zero, counts/g soil RS85	Increase[a] (fold) in count after addition of:			
		water only	*A. globiformis* GF3RP		487
Total count[b]	3.3×10^7	3.9	12.5	87.5	2250
Gram negative	6.3×10^5	9.7	22.2	1159	444
Copper-resistant[c]	1.1×10^4	10.4	177.8	1778	178
C. necator + L-2	3.6×10^2	2769[d]	101 000	74 214	15 652
Recovery of added organisms[e]	—	—	4.9	0.1	0.3

[a]As compared with value at time zero; not stated as a ratio to the increase in numbers when water alone was added to the soil for incubation.
[b]Residual of the added organism, or further multiplication of it, is not a component of the total count.
[c]Resistant to 0.01% $CuCl_2 \cdot 2H_2O$.
[d]Represents attack on indigenous prey cells.
[e]Residual of added organism, or further multiplication of it, at end of experiment.

not after succeeding additions. The lack of further buildup in predator numbers was not due to their attainment of a maximum possible number for the predators in soil. This was concluded because additions of glutamic acid instead of prey cells to soil quickly elevated the indigenous *C. necator* and strain L-2 numbers to a much higher level than was obtained by adding *A. globiformis* cells. The glutamic acid acted by serving as an alternate carbon source for growth of these nonobligate predators (Makkar and Casida 1987*b*), and for production of the growth initiation factor needed for their initiation of growth (Casida 1987).

The fact that, after the initial response, further additions of *A. globiformis* cells to soil did not provide additional increases in the numbers of *C. necator* and strain L-2 could mean that, in soil, the predators were responding only partially, if at all, to the added *A. globiformis* cells. Instead, the predators might be responding to some other bacterium that had increased its growth in response to *A. globiformis*. The response of the other bacterium to *A. globiformis* would not have to be a predatory response. One possibility is a response to some stimulatory chemical elaborated by *A. globiformis*. Actually, *A. globiformis* was previously known to have some ability of this type (Labeda *et al.* 1974). Addition of *A. globiformis* cells to soil did, in fact, markedly stimulate the apparent multiplication of another kind of bacterium, the indigenous GELRITE-hydrolyzing bacteria. *Micrococcus luteus* was the only other bacterium from among those that were tested that was stimulatory, but the numbers of this bacterium are usually low in soil.

The fact that these bacteria hydrolyzed GELRITE did not seem to be significant other than that they could be detected on media solidified with GELRITE. The reason behind the response of indigenous GELRITE hydrolyzers in soil to *A. globiformis* was unclear. They did not respond to addition to soil of pure GELRITE, various other chemicals, or *A. globiformis* culture filtrates. They did, however, respond to autoclaved cells of *A. globiformis*. When the response occurred, it apparently was not one of extensive cellular multiplication. This is concluded because it occurred so quickly, i.e., within 2.5 h. This would seem to indicate that the response actually was some kind of a breaking of dormancy of a preexisting population. It is not known whether cysts might represent the dormant form.

In soil bottles incubated 3 days at 60% of soil MHC, the increases in numbers of *C. necator*, strain L-2, total bacteria, and gram-negative bacteria in response to added GELRITE-hydrolyzing cells were about the same as when *A. globiformis* cells were added to soil instead of the GELRITE-hydrolyzing cells. Since *A. globiformis* had the ability to quickly activate a preexisting population of indigenous GELRITE-hydrolyzing cells in the soil, the responses of the other bacteria to *A. globiformis* addition to the soil may actually have been to the GELRITE-hydrolyzing cells that were responding to *A. globiformis*. Further indication this might be the case was obtained by using the procedure of adding the soil to an excess of water in flasks that were incubated by shaking for 24 h. Addition of the GELRITE hydrolyzers to soil handled in this way caused marked increases in numbers of the total bacteria, gram-negative bacteria, and copper-resistant bacterial predators (including *C. necator* and strain L-2). Addition to soil of *A. globiformis* instead of the GELRITE hydrolyzers, however, caused multiplication of only the predator bacteria. In this case, there was plenty of time for the indigenous GELRITE-hydrolyzing bacteria to become activated by the *A. globiformis* cells and then undergo some limited multiplication. This would make them susceptible to attack by the predators. There was not enough time, however, for enough multiplication to occur (in response to the indigenous GELRITE hydrolyzers) of the total and gram-negative bacteria to cause a marked increase in their counts. This could mean that, with the longer term soil incubations as in the bottles, either all of the microbial responses to *A. globiformis* were actually to the GELRITE hydrolyzers that had responded to *A. globiformis*, or only the predator responses were actually associated with the *A. globiformis* cells. An argument in favor of the former and against the latter is that *A. globiformis* did not die back in the soil flasks but actually multiplied. Under the same conditions, the hydrolyzers decreased in numbers. Even in soil bottles with their longer term incubations, the numbers of added *A. globiformis* cells only slowly declined during prolonged incubation.

As noted earlier, sequential additions of *A. globiformis* cells to soil in soil bottles did not cause a corresponding sequential increase in bacterial predator numbers. This strengthened the argument that the predator response was to an intermediate organism, such as the hydrolyzers, instead of a direct attack on *A. globiformis*. A possible explanation was that, when *A. globiformis* was added to soil, the indigenous hydrolyzer bacteria responded to *A. globiformis* by breaking dormancy. The activated hydrolyzer bacteria then caused the multiplication of the other bacteria. However, in the process the hydrolyzer bacteria also suffered serious attack by the preda-

tor bacteria. As a result, the hydrolyzer bacteria were not able to respond again when the next addition of *A. globiformis* cells was made to the soil.

As noted above, the results of these studies suggest that *Arthrobacter* cells stimulate a breaking of dormancy by hydrolyzer cells, which in turn stimulate extensive multiplication of other bacteria, both gram positive and negative, in the soil. This overall sequence, however, appears to be usually prevented by the activity of the bacterial predators. The predator bacteria attack the hydrolyzer bacteria (and other bacteria), but not the *Arthrobacter* cells. The bacterial predators themselves, however, are held in check by the fact that they remove the hydrolyzer bacteria from major activity in the sequence. This prevents the hydrolyzer bacteria from responding further to the *Arthrobacter* species, and the other bacteria in soil from responding to the hydrolyzer bacteria. As a result, the predator bacteria do not benefit from additional numbers of these other bacteria to serve as prey cells for them.

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